LESSON 11:
SOMATIC EMBRYOGENESIS

Objective

To understand the process of a single cell or a group of cells initiating the developmental pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form complete plants (Somatic embryogenesis). Under natural conditions, this pathway is not normally followed, but from tissue cultures somatic embryogenesis occurs most frequently and as an alternative to organogenesis for regeneration of whole plants.

How are Somatic Embryos produced?

In somatic (asexual) embryogenesis, embryo-like structures, which can develop into whole plants in a way analogous to zygotic embryos, are formed from somatic tissues. These somatic embryos (SE) can be produced either directly or indirectly.

Let us now understand the two ways in which somatic embryos are generated.

In direct somatic embryogenesis, the embryo is formed directly from a cell or small group of cells without the production of an intervening callus. Though common from some tissues (usually reproductive tissues such as the nucellus, styles or pollen), direct somatic embryogenesis is generally rare in comparison with indirect somatic embryogenesis.

In indirect somatic embryogenesis, callus is first produced from the explant.

Embryos can then be produced from the callus tissue or from a cell suspension produced from that callus. Somatic embryogenesis from carrot is the classical example of indirect somatic embryogenesis and is explained in more detail below:

Indirect somatic embryogenesis in carrot (Daucus carota)

A callus can be established from explants from a wide range of carrot tissues

by placing the explant on solid medium (e.g. Murashige and Skoog (MS)) containing

2,4-D (1mg/l). This callus can be used to produce a cell suspension by placing it in agitated liquid MS medium containing 2,4-D (1mg/l). This cell suspension can be maintained by repeated subculturing into 2,4-D-containing medium. Removal of the old 2,4-D-containing medium and replacement with fresh medium containing abscisic acid (0.025mg/l) results in the production of embryos (Box 1.).

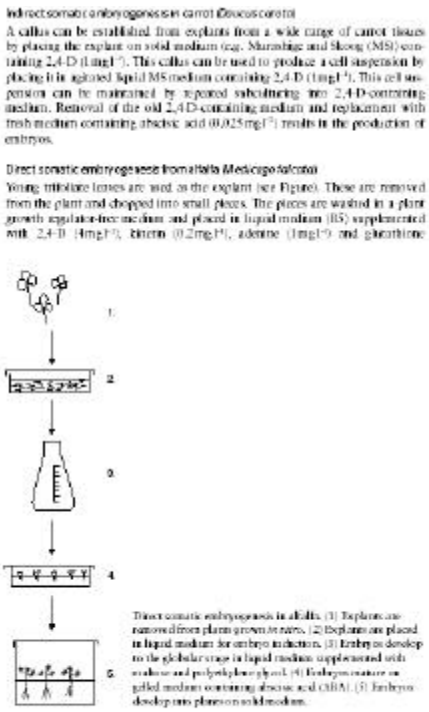
Direct somatic embryogenesis from alfalfa (Medicago falcata)

Young trifoliate leaves are used as the explant. These are removed from the plant and chopped into small pieces. The pieces are washed in a plant growth regulator-free medium and placed in liquid medium (B5) supplemented with 2,4-D (4mg/1), kinetin (0.2mg/1), adenine (1mg/1) and glutathione.

Direct somatic embryogenesis in alfalfa (Box 1.)

1. Explants are removed from plants grown in vitro
2. Explants are placed in liquid medium for embryo induction.
3. Embryos develop to the globular stage in liquid medium supplemented with maltose and polyethylene glycol.
4. Embryos mature on gelled medium containing abscisic acid (ABA).
5. Embryos develop into plants on solid medium.

Box. 1. Somatic Embryogenesis


Indirect somatic embryogenesis in carrot (Daucus carota)
A callus can be established from explants from a wide range of carrot tissues by placing the explant on solid medium (e.g. Murashige and Skoog (MS)) containing 2,4-D (1 mg l⁻¹). This callus can be used to produce a cell suspension by placing it in agitated liquid MS medium containing 2,4-D (1 mg l⁻¹). This cell suspension can be maintained by repeated subculturing into 2,4-D-containing medium. Removal of the old 2,4-D-containing medium and replacement with fresh medium containing abscisic acid (0.025 mg l⁻¹) results in the production of embryos.
Direct somatic embryogenesis from alfalfa (Medicago falcata)
Young trifoliate leaves are used as the explant (see Figure). These are removed from the plant and chopped into small pieces. The pieces are washed in a plant growth regulator-free medium and placed in liquid medium (B5) supplemented with 2,4-D (4 mg l⁻¹), kinetin (0.2 mg l⁻¹), adenine (1 mg l⁻¹) and glutathione
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Direct somatic embryogenesis in alfalfa. (1) Explants are removed from plants grown in vitro. (2) Explants are placed in liquid medium for embryo induction. (3) Embryos develop to the globular stage in liquid medium supplemented with maltose and polyethylene glycol. (4) Embryos mature on gelled medium containing abscisic acid (ABA). (5) Embryos develop into plants on solid medium.

Developmental Patterns of SEs

Somatic embryogenesis usually proceeds in two distinct stages. In the initial stage (embryo initiation), a high concentration of 2,4-D is used. In the second stage (embryo production) embryos are produced in a medium with no or very low levels of 2,4-D. In somatic (asexual) embryogenesis, embryo-like structures, which can develop into whole plants in a way analogous to zygotic embryos, are formed from somatic tissues. SEs generally originate from single cells which divide to form a group of meristematic cells. Usually, this multicellular group becomes isolated by breaking cytoplasmic connections with the other cells around it and subsequently by cutinization of the outer walls of this differentiating cell mass. The cells of meristematic mass continue to divide to give rise to globular

(round ball shaped), heart- shaped, torpedo and cotyledonary stages (Fig. 1.). In general, the essential features of SE development, especially after the globular stage, are comparable to those of zygotic embryos. Somatic embryos are bipolar structures in that they have a radicle and a plumule. The radicular end is always oriented towards the center of callus or cell mass, while the plumular end always sticks out from the cell mass. In contrast, a shoot bud is monopolar as it does not have a radicular end. In many SEs, radicle is suppressed so that they often do not produce roots; in such cases, roots have to be regenerated from the shoots produced by germinating SEs.

SEs often show abnormal developmental features, e.g., 3 or more cotyledons, bell- shaped cotyledon, larger size etc. These problems are often overcome by the presence of ABA or a suitable concentration of mannitol. In some species, normal looking somatic embryos are formed but they fail to germinate. The SEs regenerating from explant or callus are termed as primary somatic embryos. In many cases, SEs regenerate from the tissues of other SEs or the parts of germinating SEs; such SEs are called secondary somatic embryos.

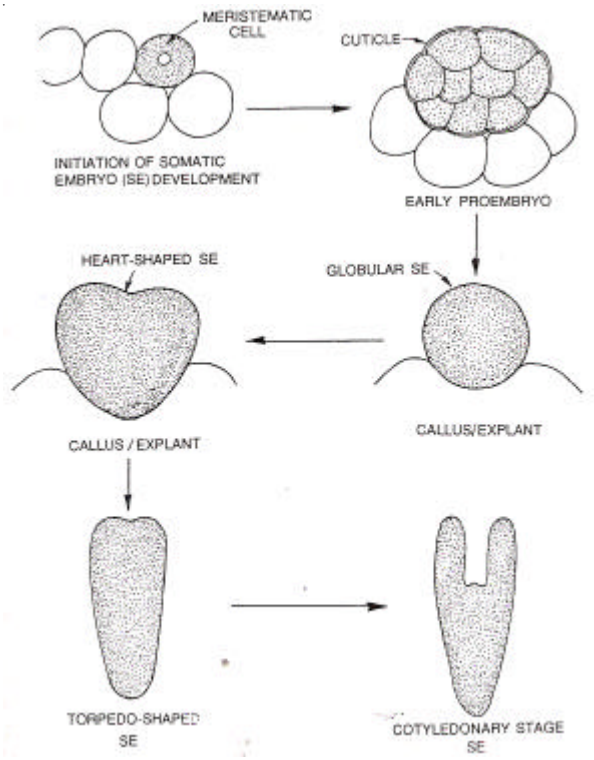

Fig. 1. Development of a Somatic embryo from a single superficial cell of the explant.

Somatic embryogenesis is influenced by several factors:

1. Growth Regulators

In most species an auxin (generally 2,4-D at 0.5- 5mg/l) is essential for somatic embryogenesis. The auxin causes dedifferentiation of a proportion of cells of the explant which begin to divide. In carrot, these small, compact cells of the explant divide asymmetrically, and their daughter cells stick together to produce cell masses called proembryogenic masses or embryogenic clumps (ECs). In the presence of auxin, the ECs grow and break up into smaller cell masses which again produce ECs. But when the auxin is either removed or reduced (0.01-0.1 mg/l) and cell density is lowered, each EC gives rise to few to several SEs; each SE is believed to develop from a single superficial cell. The ability to regenerate SEs, i.e., totipotency, is acquired by cells during dedifferentiation in response to high auxin treatment. High auxin prevents its own polar transport. 2,4-D is the most commonly used auxin in somatic embryogenesis, the others are 2,4,5-T, picloram, Dicamba etc. There are exceptional cases like carrot, where auxin is not required for somatic embryogenesis and its presence can inhibit the process. Auxins promote hypermethylation of DNA which may have a role in totipotency acquisition.

Somatic embryogenesis is a two step process:

1. SE induction on a high auxin (upto 40-60mg/l 2,4-D)
2. SE development on a low auxin or GR- free medium

In the SE induction phase, explant cells differentiate, become totipotent and, in many cases, form embryogenic clumps (ECs). Cells can be maintained in embryogenic stage on the induction medium for prolonged periods (over 10 years in carrot).

When ECs are transferred from induction medium to an appropriate medium, SE differentiation proceeds from globular, heart- shaped, torpedo to cotyledonary stages. This is called SE developmental phase. In most cases, SEs begin to germinate immediately after the cotyledonary stage. This is called SE conversion. But, often the plantlets so obtained are rather weak. It is, therefore, desirable to subject SEs to a maturation phase following their development. In this phase, the SEs usually do not grow, but undergo biochemical changes to become more sturdy and hardy. SE maturation is achieved by culturing them on a high sucrose (upto 6% or even 40%) medium or in presence of a suitable concentration (0.2-0.4 mg/l) of ABA, or by subjecting them to desiccation (usually achieved by enclosing SEs in sterile, sealed and empty petridishes). The role of Cytokinins in these systems is not known.

In some species, SEs regenerate superficially from the cotyledons and hypocotyls of developing SEs and even from germinating SEs and plantlets. This is termed as secondary embryogenesis or recurrent embryogenesis.

2. Nirogen Source

The form of nitrogen has a marked effect on somatic embryogenesis. In carrot, NH_{4+} has a promotive effect on SE regeneration. In fact, induction of SEs in carrot occurs only when about 5 m mol/kg of cell fresh weight NH_{4+} is present in the cells. This level of endogenous NH_{4+} is reached with only 2.5m mol/l of exogenous level of NH_{4+}, while 60m mol/l NO_{3-} is needed for the same. Therefore, the presence of a low level of NH_{4+} (in carrot 10m mol/l is optimal) in combination with NO_{3-} is required for SE regeneration. In carrot, NH_{4+} is essential during SE induction, while SE development occurs on a medium containing NO_{3-} as the sole nitrogen source.

3. Genotype of the Explant:

Explant genotype has a marked influence on SE regeneration. Strong genotypic effects have been shown in many species, e.g., alfalfa, wheat, maize, rice, chickpea etc. where individual genes affecting SE regeneration have been identified. Variation for regeneration ability is mainly additive and highly heritable in maize, rice and wheat, but in barley, dominance seems to be more important. In case of wheat, rice and maize, cytoplasm has a strong influence on regeneration. In some cases, this effect appears to be associated with mitochondrial genome or even the GR regime during culture. It may, therefore be postulated that at least a part of the genotypic effect on regeneration may be concerned with endogenous GR levels and/or sensitivity to exogenous GRs.

4. Other Factors

Certain other factors, such as high K^+ levels and low dissolved Oxygen levels promote SE regeneration in some species. In some other species, e.g., Citrus medica, somevolatile compounds like ethanol inhibit SE regeneration. In soyabean, low sucrose concentrations (5 and 10 g/l) promote SE regeneration as compared to high concentrations (20 and 30 g/l). In alfalfa, use of maltose as carbon source improves both SE induction and maturation (including germination) as compared to those on sucrose.

Conclusion

Somatic embryogenesis is thus, an alternative to organogenesis for regeneration of whole plants. Adherence to this pattern of morphogenesis depends on co-ordinated behaviour of a cell or cells to establish polarity as a unit and thereby initiate gene action sequentially specific to emerging tissue regions. With this method, a complete sexual apparatus is not an essential prerequisite for embryogeny in tissue cultures. In addition, somatic embryogenesis with so many practical applications (production of artificial seeds, synthesis of secondary metabolites, etc.) remains an effective tool in plant tissue culture (discussed in the next Lesson).

Questions

1. What are the two ways of somatic embryo production? Explain with examples.
2. Discuss the developmental patterns of somatic embryos with diagram.
3. Describe the cultural requirements for somatic embryo production.

Note